BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

STIC-ILL

(mc)
RB1.A4

From: Canella, Karen
Sent: Wednesday, May 14, 2003 3:05 PM
To: STIC-ILL
Subject: ill order 09/230,955

Art Unit 1642 Location 8E12(mail)

Telephone Number 308-8362

Application Number 09/230,955

1. American Journal of Pathology:
   1993 Feb, 142(2):403-412
   1993, 143(4):1150-1158
   1984, 114(3):454-460
   1996, 148(3):865-875
   1965 Sep, Vol. 44, pp. 280-282

2. Cancer Research, 1993 May 15, 53(10 suppl):2287-2299

3. Cancer epidemiology, biomarkers and Prevention, 1996 Jul, 5(7):549-557

4. Lab Investigation:
   1980, 42(1):91-96
   1988, 58(2):141-149

5. Gynecol Oncol, 1982, 13(1):58-66

6. International Journal of Gynecological Pathology:
   1985, 4(4):300-313
   1986, 5(2):151-162
   1992, 11(1):24-29

7. Differentiation:
   1986, 31(3):191-205
   1988, 39(3):185-196

8. Cancer (Phila), 1989, 63(7):1337-1342

9. Cancer Res, 1990, 50(16):5143-5152

10. Virchows Arch B Cell Pathol Incl Mol Pathol, 1987, 54 (2):98-110

11. Acta Histochemica et Cytochemica:
    1994, 27(3):251-257
    1996, 29(1):51-56

12. Archives of Gynecology and Obstetrics, 1989, 246(4):233-242

13. Clin Lab Med, 1995 Sep, 15(3):727-742

14. Clin Obstet Gynaecol, 1984 Apr, 11 (1):5-23

American Journal of Pathology, Vol. 142, No. 2, February 1993

Expression of Keratins 1, 6, 15, 16, and 20 in Normal Cervical Epithelium, Squamous Metaplasia, Cervical Intraepithelial Neoplasia, and Cervical Carcinoma

Frank Smedts,* Frans Ramaekers,† Rudolf E. Leube,‡ Karel Keijser,§ Monique Link,‖ and Peter Vooijs‖

From the Department of Pathology, Diagnostic Centre S.S.D.Z. Delft, The Netherlands; Department of Molecular Cell Biology and Genetics,† University of Limburg, Maastricht, The Netherlands; Institute of Cell and Tumor Biology,‡ German Cancer Research Centre, Heidelberg, Germany; Departments of Gynecology and Obstetrics,§ and Pathology,‖ University Hospital, Nijmegen, The Netherlands*

Expression of keratins 1, 6, 15, 16, and 20 was examined in normal cervical epithelia, squamous metaplasia, various grades of cervical intraepithelial neoplasia, and both squamous cell carcinomas and adenocarcinomas of the cervix with monospecific antibodies. Ectocervical epithelium contains all of these keratins except keratin 20. They show a heterogeneous distribution, with a basally restricted detection of keratin 15. Endocervical columnar cells were found to contain significant amounts of keratin 16, whereas the subcolumnar reserve cells expressed considerable amounts of keratin 15 and 16, and frequently keratin 6. These reserve cell keratins were also found in immature and mature squamous metaplastic epithelium. In the cervical intraepithelial neoplastic lesions they were generally found with increasing intensity as the severity of the lesion progressed. In the keratinizing variety of squamous cell carcinoma of the cervix, these three keratins seem to constitute an important part of the intermediate filament cytoskeleton, whereas in nonkeratinizing squamous cell carcinoma, they occur to a much lesser extent. Surprisingly, these keratins were also occasionally found in adenocarcinomas. From these data we conclude that the keratin phenotype of reserve cells and endocervical columnar cells is more complex than previously suggested. In particular, the keratins occurring in reserve cells are also present in most of the premalignant and in a considerable number of the malignant lesions of the cervix. The differentiation features of the various carcinoma types are, however, reflected in their specific keratin filament composition. (Am J Pathol 1993, 142:403–412)

Human keratins comprise a family of at least 20 separate intermediate filament proteins,[1,2] numbered from 1 to 20, which are distributed in a tissue-specific fashion throughout epithelia. By using specific monoclonal antibodies directed against individual keratin polypeptides, it is possible to study changes in the type of epithelial differentiation in premalignant and malignant cervical lesions. The keratin phenotypes of the various normal epithelia lining the uterine cervix as well as those of the aforementioned lesions are known to a large extent.[3–9] One of the objects of these studies has been to associate the keratin expression patterns with the metaplastic or malignant potential of cervical epithelia. Suggestions have been made by which the progressive or regressive nature of cervical intraepithelial neoplasia (CIN) may be predicted on the basis of keratin expression patterns. The expression patterns of the various cervical epithelia as currently known are shown in Figure 1.

Recently, polyclonal and monoclonal antibodies against keratins 1, 6, 15, 16, and 20[1,10–12] have become available. Of these, the antibodies to keratins 1, 6, and 16 have been tested in cervical carcinomas,[5] but, as yet, not in normal cervix or in metaplastic or CIN lesions. The observations in cervical

Accepted for publication July 23, 1992.

Address reprint requests to Dr. F. Smedts, Department of Pathology, Diagnostic Centre S. S. D. Z., Reinier De Graafweg 7, 2600 GA Delft, The Netherlands.

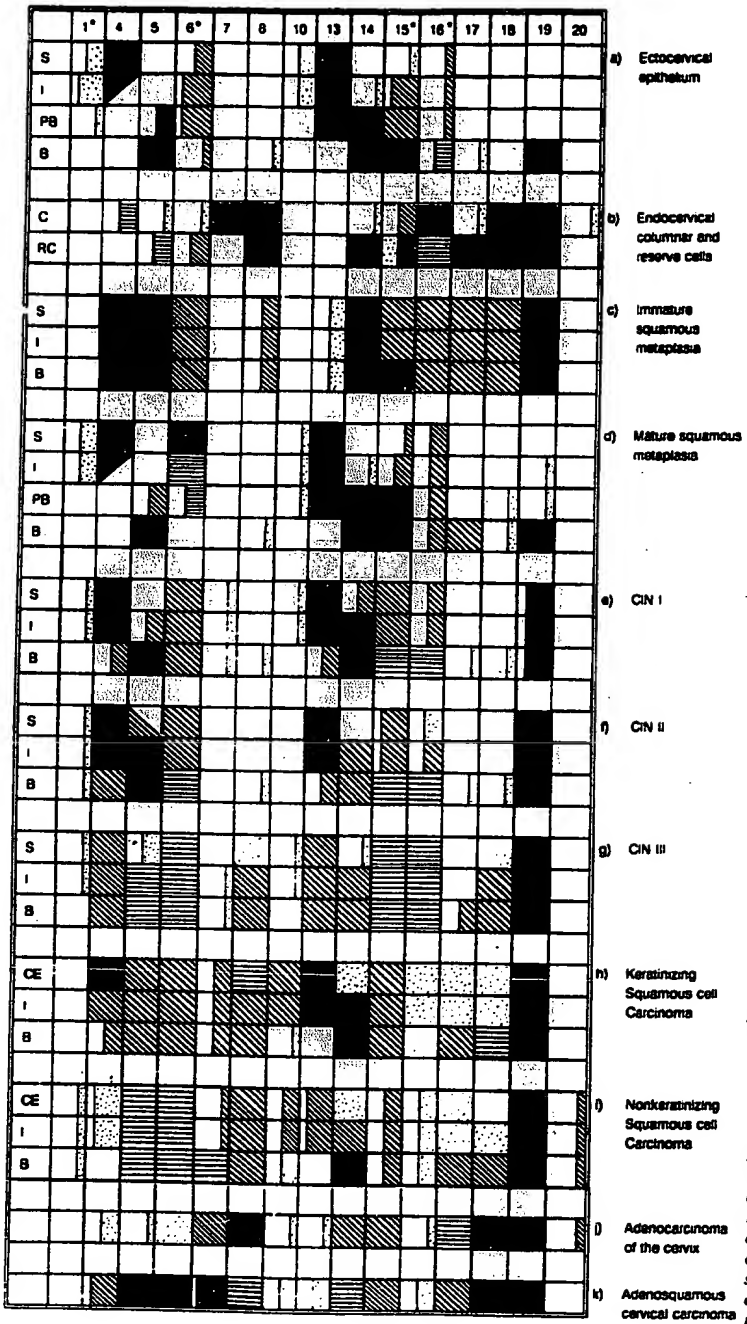

Figure 1. *Schematic representation of keratin expression patterns in normal, premalignant, and malignant cervical tissues. The human keratins are indicated by their respective numbers according to the Moll catalogue.[1,2] The asterisks indicate the keratins examined in this study. The percentage of cases staining is indicated as follows. A completely filled bar indicates that between 75 and 100% of the cases stain. A bar three-fourth filled indicates that between 50 and 75% of cases stain. A bar one-half filled denotes staining between 25 and 50% of cases. A bar one-quarter filled indicates that less than 25% of cases stain. An open bar indicates that no cases stain. A black bar indicates that all cells stain intensely; horizontal lines indicate that most cells stain and that the intensity is weak to moderate. Hatched lines mean that dispersed cells stain weak to moderately with also negative cells. Dots denote only scattered positive cells. S, superficial cells; I, intermediate cells; B, basal cells; PB, parabasal cells; C, columnar cells; RC, reserve cells; CE, central cells.*

carcinoma, the fact that keratin 15 is known to indicate metaplasia in the lung,[10,11,13] and the suggestion that keratin 16 is a marker of hyperproliferation justify a study of the expression of these keratins in cervical tissue. Furthermore, we think that an extension of our studies on keratin expression in the cervix may enhance our understanding of the processes governing epithelial metaplastic and malignant transitions.

Materials and Methods

Tissues

The tissue specimens used in this study were taken from diathermy loop excision specimens or cervical cone biopsies of 70 women with cytologically verified dysplasia. The tissue specimens representing cervical carcinomas were taken from hysterectomy specimens or biopsies of 31 women with cervical

carcinomas. The diathermy loop excision specimens, cone biopsies, and hysterectomy specimens were brought to the pathology department immediately after removal, where a small tissue fragment was removed from each specimen and snap-frozen in liquid nitrogen or liquid nitrogen-cooled isopentane. The major part of the specimen was further processed through paraffin after routine fixation in 4% buffered formalin for light microscopic analysis. A total of 98 specimens were used representing normal epithelia, metaplastic lesions, the three grades of CIN, and cervical cancer. Often, more than one type of epithelium was detected in the same fragment. In this way, normal ectocervical epithelium was diagnosed in 32 fragments, columnar endocervical epithelium in 54 fragments, reserve cells in 25 fragments, immature squamous metaplasia in 14 fragments, mature squamous metaplasia in 19 fragments, CIN I in 6 fragments, CIN II in 16 fragments, CIN III in 21 fragments, keratinizing squamous cell carcinoma in 5 fragments, nonkeratinizing squamous cell carcinoma in 13 fragments, adenocarcinoma in 10 fragments, and adenosquamous carcinoma in 3 fragments. These diagnoses were confirmed by comparison with companion formalin-fixed, paraffin-embedded preparations. Furthermore, the subepithelial connective tissue was meticulously examined for keratin expression in all fragments.

Antibodies

The specificity of the five antibodies used in this study is described as follows: 1) AF 87 is a polyclonal rabbit antiserum raised against a synthetic carboxy-terminal peptide of keratin 1. In immunoblotting studies, keratin 1 was specifically recognized. The antibody was a gift from Dr D. Roop (National Institutes of Health, Bethesda, MD). 2) AF 124 is a polyclonal rabbit antiserum raised against a synthetic carboxy-terminal peptide of keratin 6. In immunoblotting studies, only keratin 6 is recognized (S. H. Yuspa, personal communication). The antibody was a gift from Dr D. Roop. 3) Gp 15.1 are guinea pig antibodies from an animal immunized with an affinity-purified fusion protein from *Escherichia coli* containing the carboxy-terminus of human keratin 15. This antiserum reacts specifically with human keratin 15 in immunohistochemistry and immunoblotting.[10,11] 4) LL025, which recognizes keratin 16, was raised against a synthetic peptide corresponding to the carboxy-terminus of human keratin 16[14] (courtesy of Dr. E. B. Lane, Dundee, UK).[5] K20 has been shown to exclusively react with human keratin 20 in immunoblotting studies and was obtained from Sambio B. V., Uden, The Netherlands. It stains, among others, columnar cells lining the digestive tract.[1,12] As a positive control, we tested the reactivity of this antibody on normal colon epithelium.

Immunohistochemistry

Cryostat sections were fixed at room temperature in acetone for 10 minutes, air-dried, and incubated for immunohistochemical detection of keratin as previously described.[3] Both positive and negative control experiments were performed in parallel.

Results

Figure 1 shows a schematic representation of the reactivities of the keratin antibodies used in this study and those previously described in the various cervical tissues. The keratin antibodies used for the first time in this study did not react with cells in the subepithelial connective tissue.

Ecto- and Endocervical Epithelium

The basal cell compartment of ectocervical nonkeratinizing squamous epithelium was always negative for keratin 1, while in the parabasal cell compartment dispersed cells stained weakly in only 4 cases. Also, in the intermediate and superfical layers, a few dispersed cells reacted in a number of cases (Figure 2a). Keratin 6 was found extensively in the superfical layers of all but 2 cases. Basal cells were found positive in 7 cases, parabasal cells in 18 cases, and intermediate cells in 20 cases, staining with weak to moderate intensity (Figure 2b). Keratin 15 was present in the basal cells of all cases (Figure 2c), whereas in 28 cases, the parabasal compartment was positive with varying intensity, also showing negative areas. Dispersed decoration was found in the intermediate cell compartment of 20 cases, although the intensity of antibody staining and the number of positive cells was considerably less. The superficial compartment was usually completely negative, and only 4 specimens showed a weak reaction. Keratin 16 was present in the basal cell compartment of half the cases studied (Figure 2d). The above lying layers were usually negative, but in 5 specimens variable and sometimes strong staining was found through the full thickness of the epithelium. Sixteen cases were completely negative. Keratin 20 was not detected in ectocervical squamous epithelium.

The columnar cells (Figure 1b) lining the endocervical canal did not contain keratin 1. Keratin 6 was detected in less than 1% of cells in only 5 specimens (Figure 2e). Keratin 15 was not found in 40 cases,

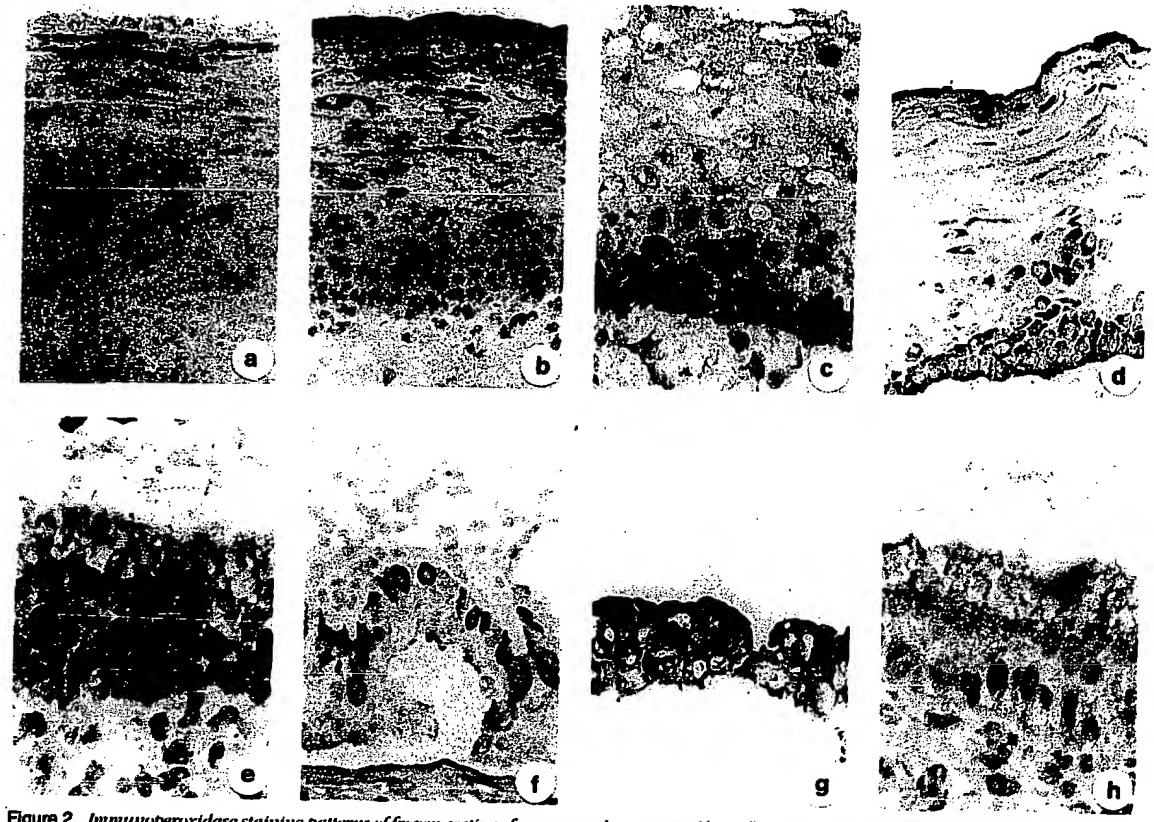

Figure 2. *Immunoperoxidase staining patterns of frozen sections from normal ectocervical* (a *to* d) *and endocervical* (e *to* h) *epithelium with reserve cells, stained with:* (a) *AE87 (keratin 1);* (b *and* e) *AE124 (keratin 6);* (c *and* f) *K15 (keratin 15);* (d *and* g) *LL025 (keratin 16);* (h) *K20 (keratin 20). Original magnification:* a *to* d, ×250; e *to* h, ×400.

whereas in 14 fragments up to 25% of the cells displayed weak to moderate positivity (Figure 2f). Keratin 16 was detected in virtually all columnar cells (Figure 2g) and keratin 20 was weakly detectable in approximately 5% of cells in only 5 cases. In general, however, columnar cells were not decorated.

Reserve cells (Figure 1b) did not contain keratin 1. In dispersed cells of 5 cases, keratin 6 could be detected (Figure 2e); in 8 cases all reserve cells stained with the keratin 15 antibody. In the other cases, approximately one-half of the reserve cells weakly expressed keratin 15 (Figure 2f). Keratin 16 was found in all reserve cells showing moderate positivity (Figure 2g). Keratin 20 was not detected in reserve cells (Figure 2h).

Immature Squamous Metaplastic Epithelium

Keratin 1 was not detected in this type of epithelium (Figure 3a), and the keratin 6 antibody showed weak to moderate positivity in 10 cases with roughly 50% positive cells, irregularly distributed through the epithelium (Figure 3b). In 2 fragments, only a few cells seemed to express keratin 6. Two cases were completely negative. Keratin 15 was found in all cases with the majority of cells positive. The intensity of immunostaining and the number of reactive cells was usually highest in the basal cell layers. In the superficial cell layers, there were both positive and negative cells (Figure 3c). Keratin 16 was weakly to moderately expressed in all cells of 7 cases (Figure 3d), and in 2 cases between 25 and 75% of the cells were weakly positive. In one case, only a few cells stained, and one case was completely negative. Keratin 20 was not found in immature squamous metaplastic epithelium.

Mature Squamous Metaplastic Epithelium

As expected, the keratin phenotype of this type of epithelium was much the same as described for ectocervical epithelium. This was definitely the case for keratins 1, 6, 15, and 20. Keratin 16, however, showed a less predictable staining pattern, as in 2 cases the full thickness of the epithelium displayed an intense immunoreaction, 2 cases showed positively in the majority of cells through the full thickness of the epithelium and 5 cases displayed a variable staining pattern. The other 10 cases were completely negative.

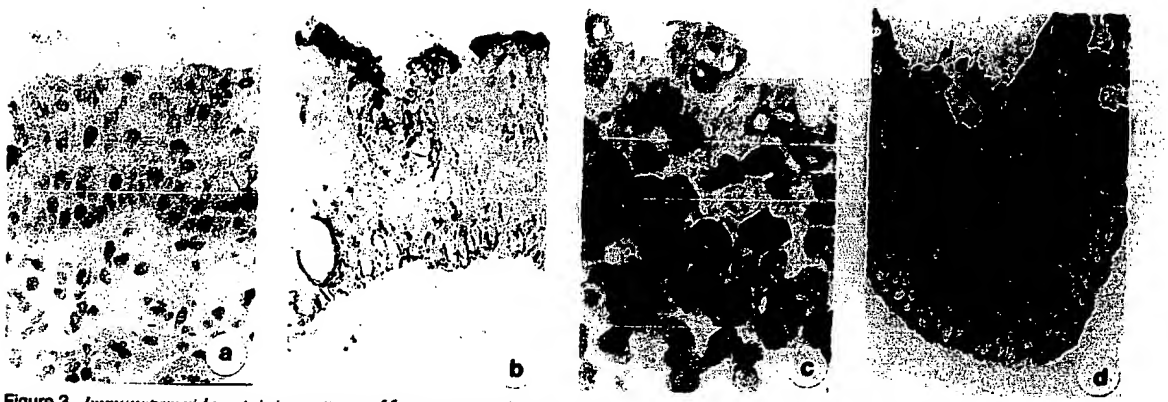

Figure 3. *Immunoperoxidase staining patterns of frozen sections from immature squamous metaplasia after staining with:* (a) *AF87 (keratin 1);* (b) *AF124 (keratin 6);* (c) *K15 (keratin 15);* (d) *LL025 (keratin 16). Original magnification:* a, b, d, ×300; c, ×400.

CIN I

Keratin expression in CIN I was in general less consistent than previously described for the non-neoplastic lesions. Keratin 1 antibody was usually not found to react (Figure 4a). Keratin 6 was found in all cases. About half of the cells stained with varying intensity and location (Figure 4b). Keratin 15 was found throughout in the full thickness of the epithelium in 3 cases, with moderate intensity of staining. In the other 3 cases, both negative and positive cells were found (Figure 4c). The Keratin 16 antibody was moderately positive in most cells of the basal cell compartment in all cases (Figure 4d). In 3 cases the superficial cell compartments were completely negative, while in the other 3 cases both negative and positive areas were observed throughout the full thickness of the epithelium. Keratin 20 was not detected in CIN I lesions.

CIN II

As observed for CIN I, keratin 1 was usually not found in CIN II (Figure 4e). The Keratin 6 antibody was found to stain moderately intensely in the basal cell compartment of 14 cases (Figure 4f), with also dispersed positivity of the above-lying cells. Two cases were completely negative. Keratin 15 was detected in all basal cells, where immunostaining intensity was usually moderate (Figure 4g). In the above-lying layers, dispersed decoration was seen in about 50% of the cells in 11 specimens. In 5 fragments the cells lying above the basal layer were all negative. Keratin 16 was found diffusely in the basal cell compartment of 12 cases, and in 3 cases about half of the basal cells reacted weakly (Figure 4h), with one case negative. Keratin 20 was not found in CIN II lesions.

CIN III

Keratin 1 was found in only 4 cases with dispersed weak positivity in scattered cells lying above the basal layer (Figure 4i). One case displayed weak diffuse positivity through the full thickness of the epithelium. Keratin 6 was found irregularly distributed through the full epithelial thickness in more than half of the cells of 12 fragments. In 6 cases there was diffuse positivity (Figure 4j), with 3 cases showing scattered cells or no expression at all. Keratin 15 was detectable with moderate intensity through the full thickness of the lesion in 10 cases (Figure 4k), and in some cases diminished toward the epithelial surface. The other 11 cases showed dispersed positivity, with both keratin 15 positive and negative areas, whereas the staining intensity tended to be strongest in the basal compartment. The keratin 16 antibody showed a diffuse reaction in 11 specimens, staining the full thickness of the epithelium (Figure 4l). Eight cases displayed varying decoration, which was strongest in the basal compartment, but both staining intensity and number of positive cells decreased as the epithelial surface was approached. Staining for keratin 20 was negative in all specimens.

Keratinizing Squamous Cell Carcinoma

The expression of keratins 1, 6, and 16 in these malignancies has been previously described[5] and is presented in figure 1h. Keratin 15 was found to be diffusely distributed in all cases of keratinizing squamous cell carcinoma (Figure 5a). The intensity of staining, however, varied from weak to strong. A reaction for keratin 20 was not found in these cases.

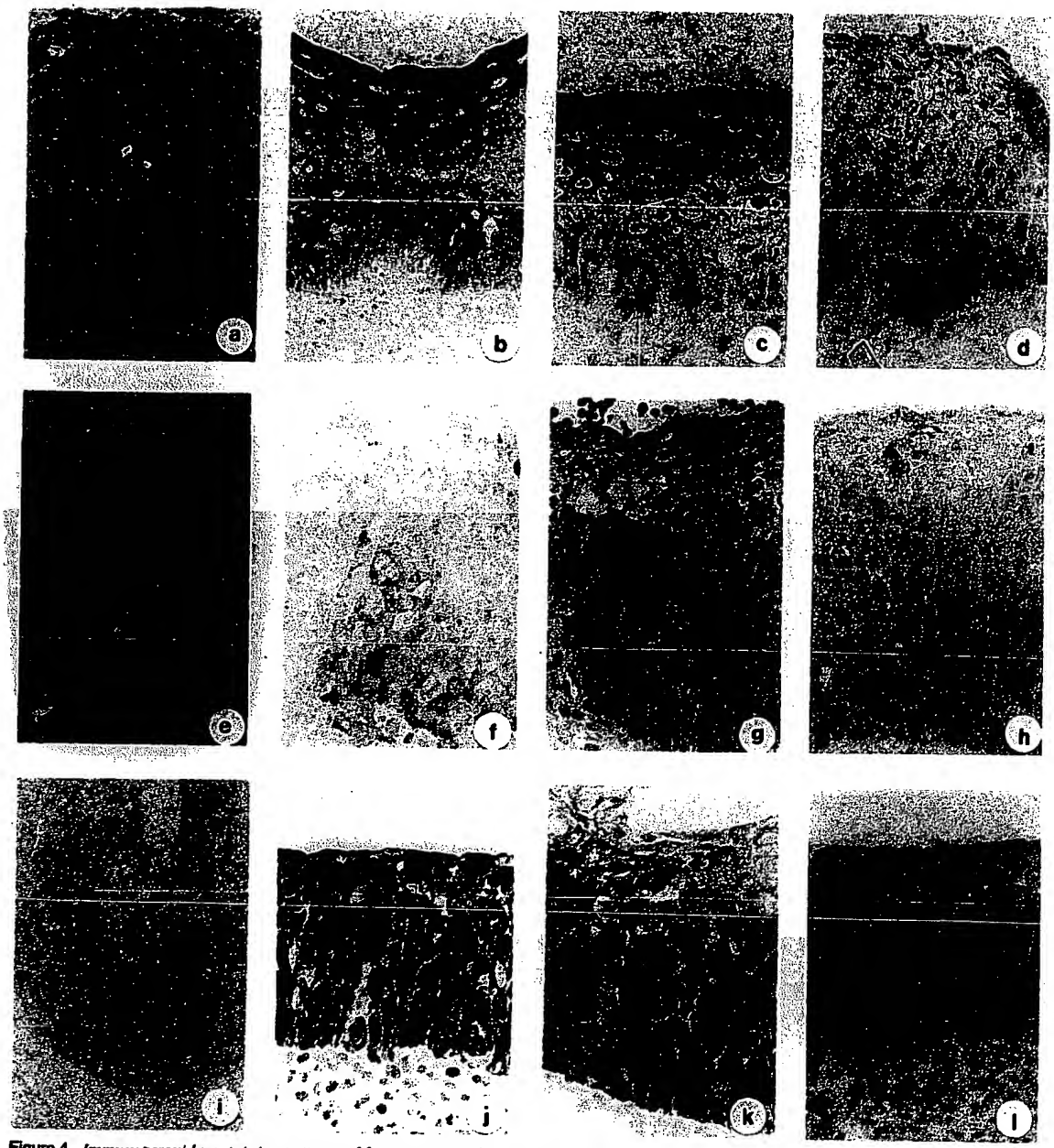

Figure 4. *Immunoperoxidase staining patterns of frozen sections from CIN I lesions* (a *to* d), *CIN II lesions* (e *to* h), *and CIN III lesions* (i *to* l) *after staining with:* (a, e, i) *AF87 (keratin 1);* (b, f, j) *AF124 (keratin 6);* (c, g, k) *K15 (keratin 15);* d, h, l) *LL025 (keratin 16). Original magnification:* a–h, ×*300;* i, ×*250.*

Large Cell Nonkeratinizing Carcinoma

Again, the previously described staining pattern of keratins 1, 6, and 16 are schematically represented in Figure 1i. Contrary to previous observations[5] we found keratin 1 to be expressed in scattered cells of 3 new fragments (Figure 5b). The intensity of the immunostaining reaction was moderate to strong, and no particular localization was noted within the tumor islands. Keratin 15 displayed an irratic staining pattern. In 4 cases strong expression was found in all cells (Figure 5c). Six cases were not stained at all. Four cases displayed a highly variable staining pattern, with negative to intensely positive areas, and the number of cells staining per fragment ranging from 5% to 60%. Keratin 20 was negative in all but one case, in which approximately 40% of the cells showed moderate staining in a scattered fashion (Figure 5d).

Adenocarcinoma

The cervical adenocarcinomas were subdivided into 5 adenocarcinomas of the endocervical type, 3 mod-

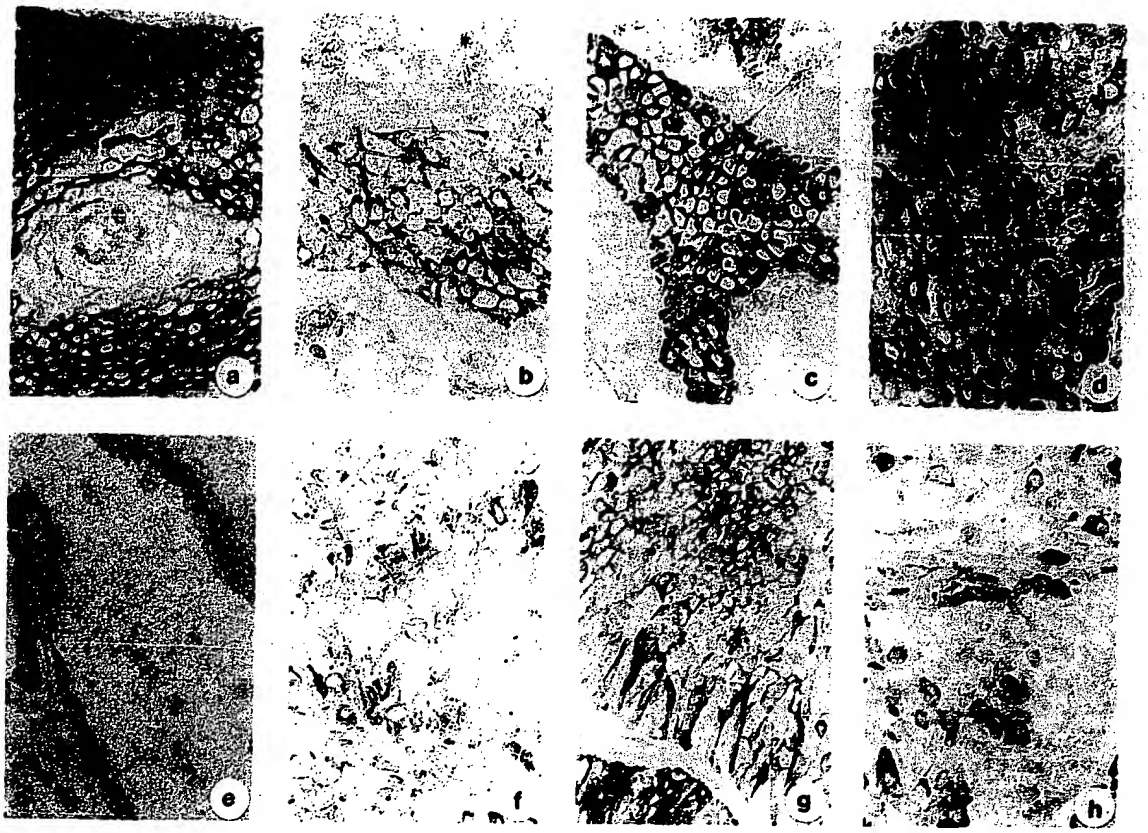

Figure 5. *Immunoperoxidase staining patterns of frozen sections from* (a) *keratinizing squamous cell carcinoma,* (b, c, d) *nonkeratinizing squamous cell carcinoma,* (e) *adenocarcinoma of the endocervical type,* (f and g) *adenocarcinoma of the endometrioid type* (h), *adenosquamous carcinoma after staining with:* (a, c, e, f, h) *K15 (keratin 15);* (b) *AE87 (keratin 1);* (d, g) *K20 (keratin 20). Original magnification:* a–h, j–l, ×300; e, ×400.

erately differentiated and 2 poorly differentiated, and 4 carcinomas of the endometrioid type, one grade I, two grade II, and one grade III (Figures 1j and 5, e–h).

Endocervical Adenocarcinoma

Keratin 15 was detected in 4 of 5 cases with a few dispersed cells staining (Figure 5e). Keratin 20 was not found in these carcinomas.

Endometrioid Adenocarcinoma

Keratin 15 was present in 3 of the 4 endometrioid adenocarcinomas, and as described in the endocervical carcinomas only a few cells stained, while the intensity varied from weak to moderate (Figure 5f).

Keratin 20 was present in 1 poorly differentiated endometrioid adenocarcinoma, with approximately 40% of cells staining with variable intensity (Figure 5g).

Adenosquamous Carcinoma

Keratin 15 was found in all 3 cases of adenosquamous carcinomas studied. In one case, all cells stained with moderate intensity (Figure 5h) in the other 2 cases a few cells expressed keratin 15. Keratin 20 was not found in adenosquamous carcinoma.

Discussion

New Aspects of Keratin Expression in the Cervix

In this study we present a number of features of keratin expression in the cervix which, to date, have not yet been described. The exact distribution of keratins 1, 6, 15, and 16 in the ectocervix is presented (for gel electrophoretic data see refs. 15 and 16). In endocervical columnar epithelium we describe the presence of keratin 16 and sporadic expression of keratins 6, 15, and 20. In regard to the keratin phenotype of endocervical reserve cells, we have shown that these contain large amounts of keratins 15 and 16 and also some weak expression of keratin 6. This means that the keratin phenotype of reserve cells is more complex than previously thought and comprises the keratins 8, 14, 16, 17, 18, and 19 in all reserve cells and frequently additional expression of keratins 5, 6, and 15. In immature and

mature squamous metaplasia,[4,6] expression of keratins 6, 15, and 16 is described. To our previous observations on the keratin expression in CIN lesions[3,4] (Figure 1) we add the expression of keratins 1, 6, 15, and 16, of which the expression of keratins 6, 15, and 16 seems to increase as the severity of dysplasia progresses (see, however, refs. 15 and 16).

In cervical carcinomas, we previously described[5] the presence of keratins 6 and 16, to which we can now add expression of keratin 15, which was always found in keratinizing squamous cell carcinoma and usually also in the nonkeratinizing variety. To the known keratin expression pattern of adenocarcinomas and adenosquamous carcinoma we add keratin 15, which is found in varying numbers of cells in most of these malignancies. In contrast to findings of Moll et al,[12] we found considerable positivity of keratin 20 in one case of squamous cell carcinoma of the cervix.

Reserve Cell Keratins Persist in Benign and Premalignant Cervical Lesions

When comparing the keratin complement of reserve cells with that of columnar cells (Figure 1), it is obvious that most keratins found in columnar cells are also found in reserve cells, with the exception of keratins 4 and 7. Also the levels of expression of several of the columnar epithelial keratins is low in reserve cells. On the basis of these findings, we hypothesize that expression of keratins 4 and 7 is initiated during the transformation of reserve cells into columnar cells, while expression of keratins 5, 6, 14, 15, and 16 is lost and expression of keratin 18 increases. Occasionally, small amounts of typical reserve cell keratins persist in columnar cells (Figure 1b and ref. 17).

When reserve cells are programmed to proliferate and differentiate into mature squamous metaplastic epithelium through immature squamous metaplasia, keratins 8 and 18 characteristic for both reserve cells and columnar cells are lost, while keratins 5, 6, 14, 15, 16, 17, and 19 become compartmentalized, showing a restricted expression to one or two epithelial layers of the metaplastic epithelium. Most important, however, is the initiation of expression of keratins 4 and 13, which are characteristic of nonkeratinizing squamous epithelia. These keratins appear during immature squamous metaplasia and become more abundant as the immature squamous metaplastic epithelium differentiates. Finally, these keratins are located above the basal cell layer in mature squamous metaplastic epithelium. Keratin 15 is characteristic of certain differentiation programs of stratified epithelia. It is co-expressed with simple epithelial keratins in basal cells of certain stratified epithelia such as in esophagus.[11] In accord with these findings is our recent observation of the varying presence of keratin 15 in cervical reserve cells, possibly indicating differences in the differentiation status of individual reserve cells. In our model those reserve cells expressing keratin 15 are predestined to progress into metaplastic epithelium. This is further supported by the observation that in reserve cell hyperplasia a considerable number of these cells contain keratin 15 and that consequently expression of this keratin in intermediate phases of squamous metaplasia and immature squamous metaplasia is considerable. In the CIN lesions keratin 15 antibodies exhibit considerable, although often erratic, staining patterns increasing with the progression of lesion.

In the case of atypical proliferation of reserve cells resulting in the development of CIN lesions, the expression of keratins 6 and 16 is of particular interest. These two keratins have been described as markers of hyperproliferation or keratinocyte differentiation in studies on regenerating epithelium of the cornea.[14] In keeping with this assumption, keratin 16 showed a restricted expression pattern in the cervix, staining approximately half of the cells in the basal cell compartment, of ectocervical epithelium, and most of the reserve cells, in line with the proliferative capacity of these cells. In our study, keratin 6 was barely detectable in these cells, whereas it showed an increasing expression, both in intensity and number of cells staining, as the superficial cell layer in the ectocervix was approached. Apparently, keratin 6 in ectocervix does not mark proliferation but seems to indicate a higher degree of differentiation. Furthermore, in the endocervical epithelium, keratin 6 did not follow keratin 16 in its expression pattern. Surprisingly, all endocervical columnar cells stained moderately with the keratin 16 antibody, thereby being the first observation of keratin 16 expression in normal columnar cells. Expression of keratins 6 and 16 increases irregularly during immature squamous metaplasia. In mature squamous metaplasia, keratin 6 expression seems to be restricted to the suprabasal cell compartment, reflecting the end stage of differentiation of this epithelium. Again, keratin 16 does not show the expected co-expression pattern with keratin 6.

With increasing grades of severity of CIN lesions, keratins 6, 15, and 16 show a progressive increase in the number of cells staining and in the staining intensity. This effect is most pronounced when CIN I and CIN II are compared with CIN III. The quantitative difference, ie, stronger staining intensity of ker-

atin 6 and 16 antibodies in CIN III, is the only feature distinguishing CIN III lesions from immature squamous metaplasia.

Reserve Cell Keratins in Cervical Carcinoma

In keratinizing squamous cell carcinoma, keratin 15 expression persists. This is not surprising, as keratin 15 has been identified as a stratification-related keratin protein. The fact that only half of the nonkeratinizing squamous cell carcinomas express keratin 15 may indicate that these carcinomas display differentiation features not discernible on light microscopic examination. In disagreement with the supposition that keratin 15 is a stratification marker is the fact that it is expressed in the majority of adenocarcinomas, albeit in a small number of cells. This, however, is further proof for the supposition that the common progenitor cell of adenocarcinomas of the cervix are the endocervical reserve cells.

Consensus Keratin Phenotypes in the Cervix

The information collected in our previous studies and extracted from the literature shows a complex keratin expression pattern in the cervix and in cervical lesions. For reasons of simplification, we previously proposed a consensus keratin phenotype for carcinomas of the cervix.[5] Such a representation indicates the presence of specific keratins within the various tissues, without giving quantitative information on the number of cells staining and the intensity of staining (Figure 6). Columnar epithelium may be distinguished from ectocervical epithelium on the basis of the absence of keratins 5, 6, 13, and 14 in the latter while simple keratins 7, 8, and 18 are present in columnar cells and not in ectocervical epithelium.

CIN I and CIN II can be distinguished from CIN III by the absence of the reserve cell keratins 8, 18, and 17. Keratinizing squamous cell carcinoma may be distinguished from nonkeratinizing carcinoma by the frequent absence of keratin 7, whereas keratins 4, 10, 13, 15, and 16, which are always present in keratinizing squamous cell carcinomas, are often absent in the nonkeratinizing type.

The complexity of the consensus keratin phenotype for adenocarcinomas of the cervix, comprising keratins 7, 8, 14, 15, 17, 18, and 19, frequently keratins 4 and 6, and sometimes keratin 5, and the similarity to the reserve cell keratin phenotype is remarkable. In our view this indicates that the adenocarcinoma keratin phenotype is more closely related to that of squamous cell carcinoma of nonkeratinizing type than previously thought.

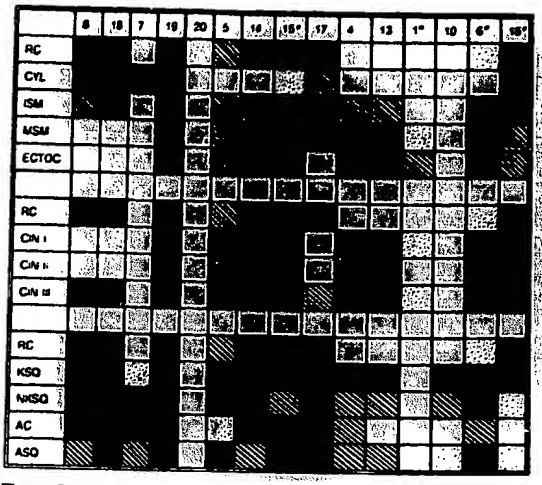

Figure 6. *Schematic representation of "consensus keratin phenotypes" of the different cervical non-neoplastic, premalignant epithelia and carcinomas. These are represented as the probability of expression of a single keratin in each of the subgroups. A completely filled box denotes a probability of expression between 75 and 100%. A hatched box denotes a probability of expression between 50 and 75%. A dotted box represents a probability of expression between 25 and 50%. An open box denotes a probability of expression between 0 and 25%. *, this study; RC, reserve cells; CYL, columnar cells; ECTOC, ectocervical nonkeratinizing squamous epithelium; ISM, immature squamous metaplastic epithelium; MSM, mature squamous metaplastic epithelium; CiN, cervical intraepithelial neoplasia; KSQ, keratinizing squamous cell carcinoma; NKSQ, nonkeratinizing squamous cell carcinoma; AC, adenocarcinoma; ASQ, adenosquamous carcinoma.*

Can the Regressive or Progressive Nature of a CIN Lesion be Predicted on the Basis of Its Keratin Expression Pattern?

It is tempting to speculate that those CIN lesions showing increased staining of the epithelium for keratins 6, 15, and 16 may represent a subfraction destined to progress into cervical cancer. Based on the findings described above, we propose the following theory for the pathogenesis of cervical cancer.

Reserve cells, containing keratins 5, 8, 14, 15, 16, 17, 18, and 19 and sometimes keratin 6, proliferate through the different grades of CIN, finally progressing into carcinoma. Those CIN lesions that maintain the keratin phenotype of the progenitor reserve cells may be destined to eventually develop into malignant lesions, whereas those losing reserve cell keratins such as keratins 8 and 18 are thought to be regressive in nature.

This is indicated by the fact that only a small number of CIN I and CIN II lesions retain the complete reserve cell keratin expression pattern. The remaining cases lose simple keratins, which means that the lesions will remain static or possibly revert into mature squamous metaplasia. In CIN III, the number

of lesions with the complete reserve cell keratin profile increased up to 50%, which percentage equals the anticipated chance of progression into cervical carcinoma from CIN III. When a CIN III lesion develops into a keratinizing carcinoma, it maintains the aforementioned keratin expression pattern and also initiates the synthesis of keratins 4, 13, and 10. In nonkeratinizing squamous cell carcinoma, the reserve cell keratins 15 and 16 are frequently lost and keratin 7 expression is acquired. If CIN III develops into an adenocarcinoma, the keratins typical of the squamous differentiation pathway are lost. Variable expression of keratin 13 in an adenocarcinoma could mean that this carcinoma represents an adenosquamous variety.

Acknowledgments

We thank Hanneke Drouen and Ton van Eupen for layout, art work, and printing the photographs. We acknowledge Marieke Konijnenburg for typographical assistance. We thank Professor M. Pruszczynski for critical comments helpful in the preparation of the manuscript.

References

1. Moll R, Schiller DL, Franke WW: Identification of protein IT of the intestinal cytoskeleton as a novel type I cytokeratin with unusual properties and expression patterns. J Cell Biol 1990, 111:567–580
2. Moll R, Franke WW, Schiller DL, Geiger B, Krepler B: The catalog of human cytokeratins: patterns of expression in normal epithelia tumors and cultured cells. Cell 1982, 31:11–24
3. Smedts F, Ramaekers F, Robben H, Pruszczynski M, Muijen G van, Lane B, Leigh I, Vooijs P: Changing patterns of keratin expression during progression of cervical intraepithelial neoplasia. Am J Pathol 1990, 136:657–668
4. Smedts F, Ramaekers F, Troyanovsky S, Pruszczynski M, Robben H, Lane B, Leigh I, Vooijs P: Basal cell keratins in reserve cells and a comparison with their expression in cervical intraepithelial neoplasia. Am J Pathol 1992, 140:601–612
5. Smedts F, Ramaekers F, Troyanovski S, Pruszczynski M, Link M, Lane B, Leigh I, Schijf C, Vooijs P: Keratin expression in cervical cancer. Am J Pathol 1992, 141:497–511
6. Weikel W, Wagner R, Moll R: Characterization of subcolumnar reserve cells and other epithelia of the human uterine cervix. Virchows Arch B 1987, 54:98–110
7. Gigi-Leitner O, Geiger B, Levy R, Czernobilsky B: Cytokeratin expression in metaplasia of the human uterine cervix. Differentiation 1986, 31:191–205
8. Angus B, Kiberu S, Purvis J, Wilkinson L, Horne CHW: Cytokeratins in cervical dysplasia and neoplasia: a comparative study of immunohistochemical staining using monoclonal antibodies NCL-5D3, CAM 5.2, and PKK1. J Pathol 1988, 155:71–77
9. Ivanyi D, Groeneveld E, Doornewaard G van, Mooi W, Hageman PC: Keratin subtypes in carcinomas of the uterine cervix: implications for histogenesis and differential diagnosis. Cancer Res 1990, 50:5143–5150
10. Leube RE, Rustad TJ: Squamous cell metaplasia in the human lung: molecular characteristics of epithelial stratification. Virchows Arch B Cell Pathol 1991, 61:227–253
11. Heid HW, Bartek J, Leube RE, Moll I, Kaufmann M, Franke WW: Cytokeratin 15 identifies a subset of cells in complex and stratified epithelia and tumors derived therefrom. Virchows Arch B Cell Pathol 1992 (submitted)
12. Moll R, Löwe A, Laufer J, Franke WW: Cytokeratin 20 in human carcinomas: a new histodiagnostic marker detected by monoclonal antibodies. Am J Pathol 1992, 140:427–447
13. Leube RE, Bader BL, Bosch FX, Zimbelman R, Achtstätter T, Franke WW: Molecular characterization and expression of the stratification-related cytokeratins 4 and 15. J Cell Biol 1988, 106:1249–1261
14. Wetzels RHW, Kuijpers HJH, Lane EB, Leigh IM, Troyanovsky SM, Holland R, Haelst JGM van, Ramaekers FCS: Basal cell specific and hyperproliferation-related keratins in human breast cancer. Am J Pathol 1991, 138:751–763
15. Czernobilsky B, Moll R, Franke WW: Intermediate filaments of normal and neoplastic tissues of the female genital tract with emphasis on problems of differential tumor diagnosis. Pathol Res Pract 1984, 179:31–37
16. Moll R, Levy R, Czernobilsky B, Hohlweg-Majert P, Dallenbach-Hellweg G, Franke WW: Cytokeratins of normal epithelia and some neoplasms of the female genital tract. Lab Invest 1983, 49:599–610
17. Muijen van GNP, Ruiter DJ, Franke WW, Achtstätter T, Haasnoot WHB, Ponec M, Warnaar SO: Cell type heterogenity of cytokeratin expression in complex epithelia and carcinomas as demonstrated by monoclonal antibodies specific for cytokeratins nos. 4 and 13. Exp Cell Res 1986, 162:97–113